氏　　名	ぎ　でうぉん 奇　大源
学 位 の 種 類	博士（薬科学）
学 位 記 番 号	富医薬博甲第 348 号
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教　育　部　名	富山大学大学院医学薬学教育部　博士後期課程 薬科学専攻
学位論文題目	Studies on chemical constituents of three marine sponges, *Siphonochalina siphonella* in Egypt and *Arenosclera* sp. and *Gelliodes* sp. in Vietnam （エジプト産海綿 *Siphonochalina siphonella*、及びベトナム産海綿 *Arenosclera* sp.と *Gelliodes* sp.の化学成分に関する研究）

論文審査委員

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様式３

論　　文　　要　　旨

Studies on chemical constituents of three marine sponges, *Siphonochalina siphonella* in Egypt and *Arenosclera* sp. and *Gelliodes* sp. in Vietnam

医学薬学教育部博士後期課程･薬科学専攻

奇　大源　(KI DAE-WON)

1. Introduction

Cancer is the second leading cause of death in the world. According to the 2018 reports, one in six people worldwide is reported to die as a result of cancer. The discovery of anticancer drugs has been utilized extensively, but there has been no report on excellent selective activity in cancer cells. In addition, plants have always been the main source of the anticancer drugs. Some of the acclaimed examples are vincristine and vinblastine isolated from the Madagascar periwinkle plant and taxol isolated from the western yew tree. However, marine natural products have not yet been fully exploited.

The discovery of bioactive substances from marine sponges has been the limelight in the pharmaceutical field over the past decade owing to the production of many bioactive compounds from the sponges to protect themselves against the environment. On top of that, marine sponges also produced cytotoxic compounds such as terpenoids, alkaloids, steroids, and peptides which suggests that marine sponges have high potential in the development of anticancer drugs. In the effort to search for new cytotoxic compounds, one marine sponge was collected in Egypt and seven marine sponges collected in Vietnam, respectively. The MeOH extracts from the eight marine sponges were screened for cytotoxic activities against human cervical cancer cell line (HeLa), human breast cancer cell line (MCF-7), and human lung cancer cell line (A549). Among them, Egyptian marine sponge, *Siphonochalina siphonella*, and two Vietnamese marine sponges, *Arenosclera* sp. and *Gelliodes* sp. exhibited cytotoxic activities against HeLa, MCF-7, and A549 with IC_{50} values on each cell line at 25.5, 7.9, and 36.7 μg/mL, 13.0, 8.5, and 25.4 μg/mL, 27.5, 9.0, and 32.2 μg/mL, respectively. Thus, this study aimed to search for new cytotoxic compounds from marine sponges *S. siphonella* in Egypt and *Arenoscelra* sp. and *Gelliodes* sp. in Vietnam.

2. Constituents of *Siphonochalina siphonella* collected in Egypt and their cytotoxic activities[1, 2)]

S. siphonella (syn. *Callyspongia siphonella*) belongs to the Callyspongiidae family and only exists in the Red Sea. The chemical constituents isolated from this sponge are triterpenoids, brominated oxindole alkaloids, sterols, and polyacetylenes with their reported biological activities such as anti-osteoclastogenesis, anticancer, antibacterial, and anti-inflammatory activities. Among them, polyacetylenes are one class of natural compounds with several functional groups such as amides, halogens, carbonyls and so on. Callyspongamide A, a structurally rare compound consisting of a phenyl moiety and a linear polyacetylene, has also been isolated from marine sponge *C. fistularis*, as a cytotoxic polyacetylene compound.

The investigation of the cytotoxic compounds from the $CHCl_3$ soluble fraction of the MeOH extract of *S. siphonella* led to isolation of three new polyacetylene amides, siphonellamide A (**1**), siphonellamide B (**2**), and siphonellamide C (**3**), a new monoacetylene amide, siphonellamide D (**4**), a new indole fatty amide, siphonellamide E (**5**), and three new polyacetylene alcohols, siphonellanol A (**6**), siphonellanol B (**7**), and siphonellanol C (**8**) with four known compounds, *N*-[2′′-(1′*H*-indol-3′-yl)ethyl]-hexadecanamide (**9**), callyspongamide A (**10**), dehydroisophonochalynol (**11**), and siphonochalynol (**12**) (**Figure 1**). The structures of all isolated compounds were determined by comparing their spectroscopic data with those reported in the literatures. Notably, this is the first example for the isolation of the indole acetylene amide type of compounds from natural resource.

All isolated compounds, except for **3** and **4**, were evaluated for cytotoxic activities against three cancer cell lines, HeLa, MCF-7, and A549 (**Table 1**). Polyacetylene amides **1**, **2**, and **10** exhibited cytotoxic activities against three cancer cell lines with IC_{50} values ranging from 9.4 to 34.1 μM. Polyacetylene alcohols **6**–**8**, **11**, and **12** showed cytotoxic activities against HeLa with IC_{50} values ranging from 23.9 to 26.5 μM and moderate cytotoxic activities against MCF-7 and A549. On the other hand, indole fatty amide **5** showed weak cytotoxicity against HeLa but didn't show cytotoxicities against MCF-7 and A549. Compound **9** also didn't have cytotoxicities against three cancer cell lines. Comparisons of the cytotoxic results of the isolated compounds confirmed that the acetylene moiety plays an important role in the cytotoxic activities, as previously reported. Furthermore, comparisons of the structure and activity of phenyl polyacetylene amides **1** and **10**, indole polyacetylene amide **2**, and polyacetylene alcohols **6**–**8**, **11**, and **12** suggested that the combination of the acetylene moiety with the phenyl or indole moiety could increase cytotoxic activities against MCF-7 and A549. Hence, the isolation of chemical constituents from *S. siphonella* provided insights into not only the chemodiversity of marine sponges, but also the potential of Egyptian *S. siphonella* as a natural source of cytotoxic compounds.

3. Constituents of *Arenosclera* sp. collected in Vietnam and their cytotoxic activities[3)]

Arenosclera sp. belongs to Callyspongiidae family and is widely distributed in Southwest Pacific (Australia), New Caledonia, and South China Sea. Only the bioactive substances of *A. brasiliensis* from this genus have been reported previously with four alkylpiperidine alkaloids with cytotoxic and antimicrobial activities. The investigation of the bioactive compounds from the MeOH extract of *Arenosclera* sp. led to isolation of nine polybrominated diphenyl ethers (PBDEs) (**13**–**21**) including a new PBDE, tribromoiododiphenyl ether (**13**), and three sterols (**22**–**24**) (**Figure 2**). PBDEs **13**–**21** were isolated for the first time from *Arenosclera* sp. All twelve isolated compounds were evaluated for cytotoxicity against three cancer cell lines (HeLa, MCF-7, and A549) (**Table 2**). Compound **13** showed cytotoxicity against only MCF-7 with an IC_{50} value of 35.6 μM. Compound **15** exhibited strong cytotoxicity against MCF-7, whereas **14** showed strong cytotoxicites against all three cancer cell lines. Comparisons of the cytotoxic results of **14**, **18**, and **20** suggested that the *para*-orientation relationship between the hydroxy and bromine groups at C-1 and C-4 was the most important feature for enhancing the cytotoxic activities. On the other hand, structure-activity relationship of **13**, **18**, **20**, and **21** indicated that, in the case of the absence of bromine at C-4, the presence of the iodine moiety at C-6 or the methoxy group at C-1 were crucial for enhancing the cytotoxicity. These occurrences in *Arenosclera* sp. provide insight into not only the bioactive compounds in the Vietnamese *Arenosclera* sp. marine sponge, but also the biodiversity of the *Arenosclera* species from the view point of their chemical constituents.

4. Constituents of *Gelliodes* sp. collected in Vietnam and their cytotoxic activities[4)]

Gelliodes sp. belongs to Niphatidae family and is widely spread in several regions around the Indian Ocean, West Pacific Ocean, New Guinea, New Caledonia, and South China Sea. Only twelve carotenoids including a new aldehydic carotenoid, gelliodesxanthin, have been reported from *G. callista*. The investigation of the cytotoxic substances from the MeOH extract of *Gelliodes* sp. led to isolation of a decenoic acid derivative, gelliodesinic acid (**25**), one natural new indole derivative (**26**), three known furanoterpenoids (**27**–**29**), and two known indole derivatives (**30**–**31**) (**Figure 3**). All isolated compounds were evaluated on cytotoxic activities against three cancer cell lines, HeLa, MCF-7, and A549 (**Table 3**). Furanoterpenoids **27**–**29** showed moderate cytotoxic activities against three cancer cell lines with IC_{50} values ranging from 13.2 to 75.5 μM. Compound **25** and two indole derivatives **26** and **31** didn't show cytotoxic activities. However, another indole derivative **30** revealed weak cytotoxic activity against A549 with an IC_{50} value of 89.2 μM. The results provided new insights into not only chemical components of Vietnamese *Gelliodes* sp., but also chemodiversity of furanoterpenoids and indole derivatives in marine sponges.

Conclusion

Total 31 compounds including eight new polyacetylenes (**1**−**8**), a new polybrominated diphenyl ether (**13**), a new decenoic acid derivative (**25**), together with 21 known compounds were isolated from three marine sponges, Egyptian *S. siphonella* and Vietnamese *Arenosclera* sp. and *Gelliodes* sp. All isolated compounds **1**−**31** were evaluated on cytotoxic activities against three human cancer cell lines (HeLa, MCF-7, and A549). Polyacetylenes, PBDEs, and furanoterpenoids exhibited cytotoxic activities. The results suggested that these three skeletons could be attractive candidates for the study on the cytotoxic activities. Taken together, the present study in conjugated with the cytotoxic activities against human cancer cell lines provided new insight into not only chemical components of the Egyptian and Vietnamese marine sponges, but also chemodiversity of the isolated compounds in marine sponges.

References

1. Ki DW, El-Desoky AH, Kodama T, Wong CP, Ghani MA, El-Beih AA, Mizuguchi M, Morita H (2020) New cytotoxic polyacetylene amides from the Egyptian marine sponge *Siphonochalina siphonella*. *Fitoterapia* 142:104511.
2. Ki DW, El-Desoky AH, Wong CP, Ghani MA, El-Beih AA, Mizuguchi M, Morita H (2020) New cytotoxic polyacetylene alcohols from the Egyptian marine sponge *Siphonochalina siphonella*. *J Nat Med* 74:409–414.
3. Ki DW, Awouafack MD, Wong CP, Nguyen HM, Thai QM, Ton Nu LH, Morita H (2019) Brominated diphenyl ethers including a new tribromoiododiphenyl ether from the Vietnamese marine sponge *Arenosclera* sp. and their antibacterial activities. *Chem Biodivers* 16:e1800593.
4. Ki DW, Kodama T, El-Desoky AH, Wong CP, Nguyen HM, Do KM, Thai QM, Ton Nu LH, Morita H (2020) Chemical constituents of the Vietnamese marine sponge *Gelliodes* sp. and their cytotoxic activities. *Chem Biodivers*, accepted, Doi: 10.1002/cbdv.202000303.

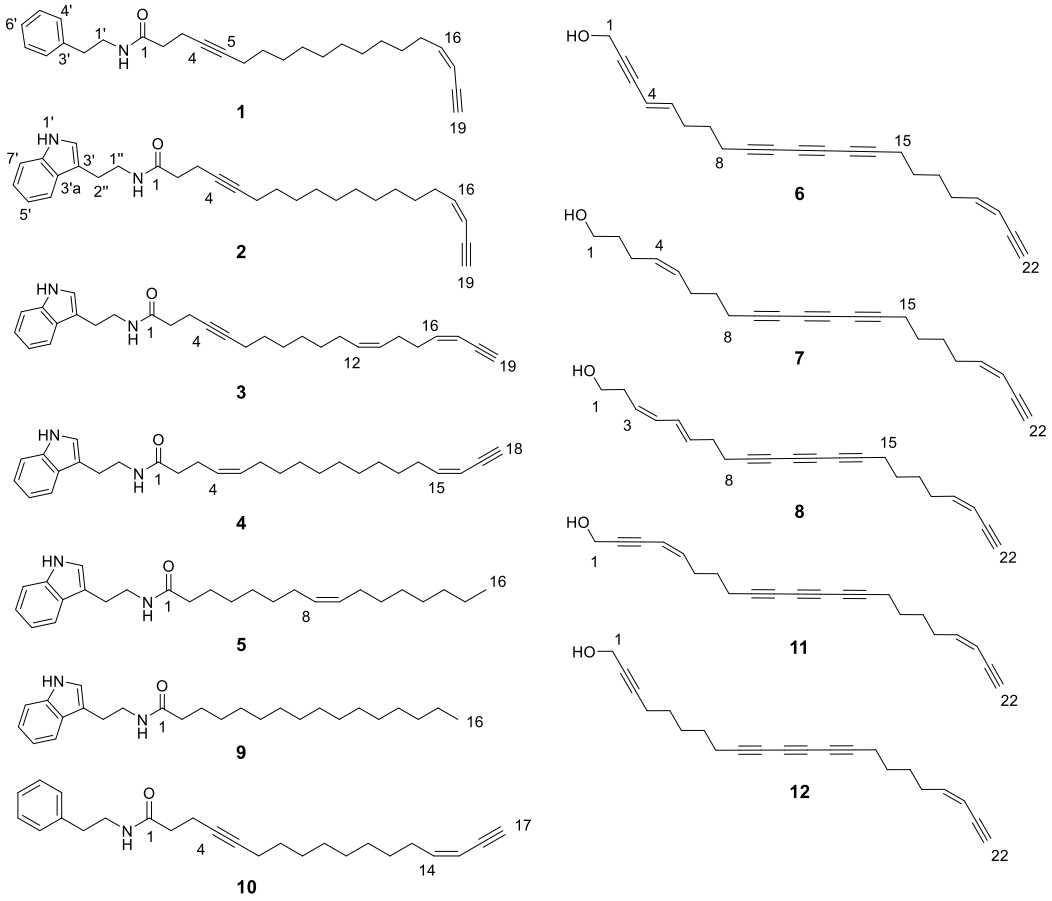
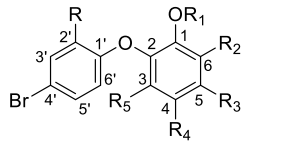


Figure 1. Structures of compounds **1**–**12** isolated from the MeOH extract of *S. siphonella*

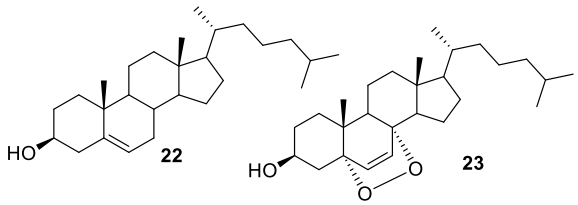
Table 1. Cytotoxic activities of **1**–**12** from *S. siphonella*

Samples	IC_{50} (μM)		
	HeLa	MCF-7	A549
1	9.4	18.0	24.2
2	17.4	34.1	25.9
5	78.4	> 100	> 100
6	26.5	54.9	59.8
7	26.2	69.2	59.9
8	25.9	57.6	58.5
9	> 100	> 100	> 100
10	25.5	19.4	30.0
11	23.9	61.4	63.4
12	25.4	61.3	60.4
5-FU [a]	28.4	34.7	14.6

[a] 5-Fluorouracil: positive control.



	R	R_1	R_2	R_3	R_4	R_5
13	Br	H	I	H	H	Br
14	Br	H	Br	H	Br	H
15	H	H	H	Br	H	Br
16	Br	H	H	Br	H	H
17	Br	H	Br	Br	H	Cl
18	Br	H	H	Br	H	Br
19	Br	H	Br	Br	H	Br
20	Br	H	Br	H	H	Br
21	Br	OCH_3	H	Br	H	Br



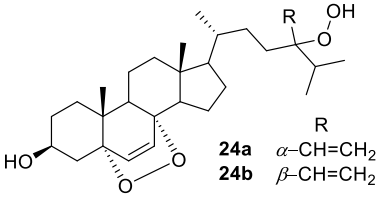


Figure 2. Structures of compounds **13**–**24** isolated from the MeOH extract of *Arenosclera* sp.

Table 2. Cytotoxic activities of **13**–**24** from *Arenosclera* sp.

Samples	IC_{50} (μM)		
	HeLa	MCF-7	A549
13	> 100	35.6	> 100
14	6.9	5.0	7.5
15	38.6	5.1	68.5
16	86.8	68.3	> 100
17	72.3	64.1	73.2
18	> 100	> 100	> 100
19	62.0	43.3	78.3
20	> 100	> 100	> 100
21	65.7	51.4	39.7
22	> 100	> 100	> 100
23	> 100	> 100	> 100
24a/b	> 100	> 100	> 100
5-FU [a]	43.1	46.2	47.5

[a] 5-Fluorouracil: positive control.

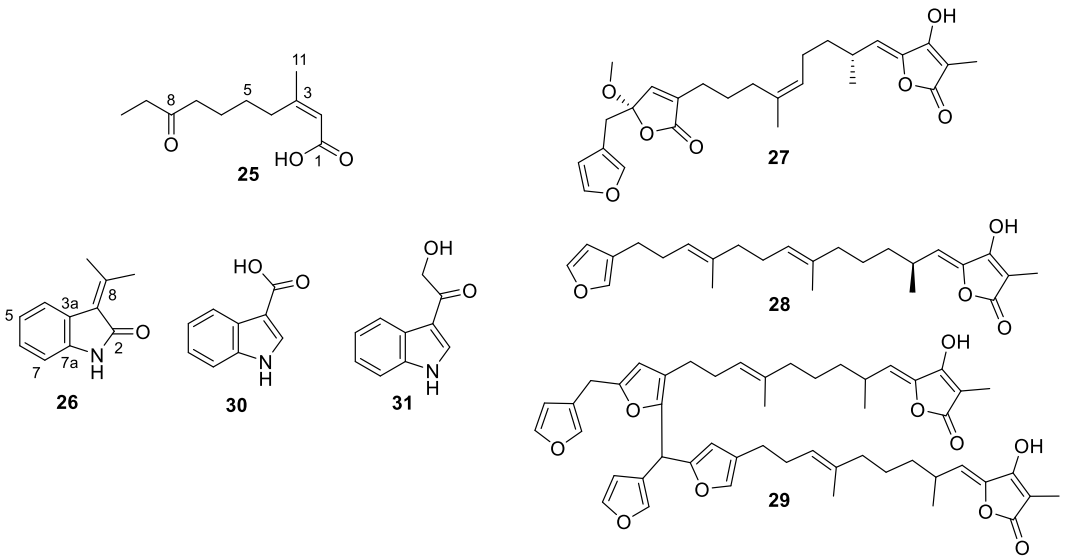


Figure 3. Structures of compounds **25**–**31** isolated from the MeOH extract of *Gelliodes* sp.

Table 3. Cytotoxic activities of **25**–**31** from *Gelliodes* sp.

Samples	IC_{50} (μM)		
	HeLa	MCF-7	A549
25	> 100	> 100	> 100
26	> 100	> 100	> 100
27	41.8	29.3	23.6
28	35.4	25.0	75.5
29	69.0	54.2	58.6
30	> 100	> 100	89.2
31	> 100	> 100	> 100
5-FU [a]	43.1	46.2	47.5

[a] 5-Fluorouracil: positive control.

様式７

学位論文審査の要旨

報告番号	富医薬博甲第　　号 富医薬博乙第　　号	氏名	奇　大源
審査委員	職名	氏名	
	（主査）　教授	松谷　裕二	印
	（副査）　教授	小松　かつ子	印
	（副査）　教授	森田　洋行	印

（論文題目）

Studies on chemical constituents of three marine sponges, *Siphonochalina siphonella* in Egypt and *Arenosclera* sp. and *Gelliodes* sp. in Vietnam

（エジプト産海綿*Siphonochalina siphonella*、及びベトナム産海綿*Arenosclera* sp.と*Gelliodes* sp.の化学成分に関する研究）

（判定）

合格

（論文審査の要旨）（２頁以内）

海綿は，強力な細胞毒性を有する二次代謝産物の供給源である。このことから，海綿の生物活性及び化学成分を評価することは，抗がん剤のシードとなり得る新規細胞毒性化合物の発見に繋がることが期待される。申請者は，がん細胞に対して細胞毒性を示す新規化合物を取得することを目的に，ヒト子宮頸癌由来HeLa細胞，ヒト乳腺癌由来MCF-7細胞，及びヒト肺胞基底上皮腺癌由来A549細胞に対して細胞毒性を示したエジプトの海綿*Siphonochalina siphonella*とベトナムの海綿*Arenosclera* sp.及び*Gelliodes* sp.のメタノール抽出物から，化学成分の単離・構造決定を実施し，31化合物を単離した。さらに，単離した2種の化合物が，ヒト由来がん細胞に対して強い細胞毒性を示すことを見いだすに至った。本研究に関する内容の骨子と審査結果は，下記に示すとおりである。

1. エジプト産*S. siphonella*の化学成分と単離した化合物の細胞毒性

*S. siphonella*のメタノール抽出物について，各種クロマトグラフィーを用いて分画・精製した。その結果，3種の新規ポリアセチレンアミド(**1–3**)，1種の新規インドール脂肪酸アミド(**4**)，3種の新規ポリアセチレンアルコール(**5–8**)を，1種の既知インドール脂肪酸アミド(**9**)，1種の既知ポリアセチレンアミド(**10**)，及び2種の既知ポリアセチレンアルコール(**11, 12**)とともに単離することに成功した。得られた化合物の化学構造は，各種スペクトルデータを詳細に解析して決定した。天然でも希な化合物であるポリアセチレンアミドが本海綿に含まれていることを示したのは，本研究が最初である。さらに，単離した**1**, **2**, 及び**5–12**のHeLa細胞，MCF-7細胞，及びA549細胞に対する細胞毒性を精査することで，**9**を除く全ての化合物が評価した全てのヒト由来がん細胞に対して細胞毒性を示すこと，及びそれらの中でも，**1**がいずれの細胞に対しても最も高い細胞毒性を示すことを明らかにした。**1**のHeLa細胞，MCF-7細胞，及びA549細胞に対するIC_{50}値は，それぞれ9.4, 18.0, 24.2 μMであった。これらの構造活性相関の解析により，ポリアセチレンへのフェニルアミドまたはインドールアミドの導入がポリアセチレンの細胞毒性を高めることを提唱した。

2. ベトナム産*Arenosclera* sp.の化学成分と単離した化合物の細胞毒性

*Arenosclerai*種の化学成分については，*A. brasiliensis*から4種の4環性アルキルピペリジンが報告されているのみである。ベトナム産*Arenosclerai* sp.のメタノール抽出物について，各種クロマトグラフィーを用いて分画･精製した結果，1つのヨウ素を置換基として有する新規ブロモ化ジフェニルエステル(**13**)を，8種の既知ブロモ化ジフェニルエステル(**14–21**)と3種の既知ステロール(**22–24a/b**)とともに単離することに成功した。得られた化合物の化学構造は，各種スペクトルデータを詳細に解析して決定した。さらに，単離した**14–21**のHeLa細胞，MCF-7細胞，及びA549細胞に対する細胞毒性を精査することで，**14–17, 19**，及び**21**が評価した全てのヒト由来がん細胞に対して細胞毒性を示すこと，及びそれらの中でも，**14**が6.9, 5.0, 7.5 μMのIC_{50}値で，HeLa細胞，MCF-7細胞，及びA549細胞それぞれに対して最も高い細胞毒性を示すことを明らかにした。これらのことから，C-1位とC-4位の水酸化と臭素化が，ジフェニルエステルの細胞毒性を高めるために必要であることを提唱した。

3. ベトナム産*Gelliodes* sp.の化学成分と単離した化合物の細胞毒性

*Gelliodes*種の化学成分については，*G. callista*から12種のカロテノイドが報告されているのみである。ベトナム産*Gelliodes sp.*のメタノール抽出物について，各種クロマトグラフィーを用いて分画･精製した結果，1種の新規デカン酸誘導体(**25**)と天然においては新規となる1種のインドール誘導体(**26**)を，3種の既知フラノテルペノイド(**27–29**)と2種の既知インドール誘導体(**30, 31**)とともに単離することに成功した。得られた化合物の化学構造は，各種スペクトルデータを詳細に解析して決定した。さらに，単離した**25–31**のHeLa細胞，MCF-7細胞，及びA549細胞に対する細胞毒性を精査することで，**27–29**が評価した全てのヒト由来がん細胞に対して弱いながらも細胞毒性を示すことを明らかにした。

以上のように，奇 大源は，エジプト産海綿1種とベトナム産海綿2種から新規化合物9種を含む31種の化合物を単離・同定し，さらに，単離した化合物3種について，ヒト由来がん細胞に対する細胞毒性を明らかにした。強い活性を示した**1**と**14**については，新たな抗がん剤のシードとなることが期待される。エジプト産海綿及び*Arenosclerai*種と*Gelliodes*種の海綿については，未だ化学成分解析が不十分であるものの，本研究結果は，海洋生物の化学成分について新たな科学的知見を与えたと言える。

主査及び副査は，論文内容と面接試験を通して，申請者 奇 大源に，博士（薬科学）の学位を授けるに値すると判定した。

（学位論文のもとになる論文　著者名, 論文題目, 掲載誌名, 巻, 最初の頁と最後の頁, 年を記載）

1. Ki DW, El-Desoky AH, Kodama T, Wong CP, Ghani MA, El-Beih AA, Mizuguchi M, Morita H, New cytotoxic polyacetylene amides from the Egyptian marine sponge *Siphonochalina siphonella*, *Fitoterapia*, **142**, 104511 (2020).
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